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The Cornified Envelope: A First Line of Defense against Reactive Oxygen Species

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Human skin serves as a barrier against multiple environmental insults, including pathogenic microorganisms, pollutants, toxic chemicals, and UV radiation. In the outermost layer of the epidermis, the cornified envelope functions as a mechanical and permeability barrier. In this issue, Vermeij *et al*. report a novel function of cornified envelope proteins as a first-line antioxidant barrier to protect the body from damage induced by reactive oxygen species.

Journal of Investigative Dermatology (2011) **131**, 1409–1411. doi:10.1038/jid.2011.119

Reactive oxygen species and the protective function of the epidermis

Reactive oxygen species (ROS) are highly reactive molecules formed in the metabolism of oxygen. Low levels of ROS are required for efficient cellular signaling. However, a variety of challenges, such as excessive cellular metabolism, inflammation, UV irradiation, and exposure to xenobiotics, can increase the levels of intra- and extracellular ROS, resulting in an impaired redox balance, defined as oxidative stress. This condition is deleterious for cells because it leads to oxidative damage to proteins, lipids, and DNA. Consequently, oxidative stress can result in apoptosis, malignant transformation, and cell aging, and it is involved in the pathogenesis of major human diseases, including neurodegenerative diseases, diabetes, atherosclerosis, and cancer. On the other hand, high levels of ROS formed by inflammatory cells are an important component of the innate immune system, required for defense against pathogens.

The epidermis is a barrier against environmental insults, including pathogens, pollutants, toxic chemicals, and UV radiation, all of which can lead—directly or indirectly—to oxidative stress. Therefore, the epidermis must cope with high levels of ROS. Cellular ROS defense mechanisms comprise ROS-detoxifying enzymes such as catalase, superoxide dismutases, various types of peroxidases, and peroxiredoxins, as well as low-molecular-weight antioxidants such as vitamins C and E, uric acid, and the tripeptide glutathione.

> Cornified envelope proteins provide protection against ROS.

Previous studies have demonstrated that suprabasal keratinocytes of the epidermis are better protected from ROS damage than basal keratinoctyes (Figure 1). Thus, differentiation of keratinocytes *in vitro* results in increased ROS-detoxifying enzyme activities and consequently reduced ROS damage (Sasaki *et al.*, 2005; Vessey *et al.*, 1995). In murine epidermis, higher expression and activity of the cytoprotective transcription factor Nrf2 in suprabasal compared with basal keratinocytes have been reported. The result is a suprabasal to basal gradient of ROS-detoxifying enzymes and of proteins involved in glutathione transport and biosynthesis. As a consequence, suprabasal keratinocytes are protected from UVB-induced ROS damage and apoptosis (Schäfer *et al.*, 2010). Furthermore, a higher expression of the 8-OHdG repair enzyme 8-oxoguanine-DNA glycosylase 1 has been identified in suprabasal layers of the human epidermis (Javeri *et al.*, 2008). The low ROS detoxification capacity of basal keratinocytes is of major biological relevance because it prevents the survival of mutated stem cells and transit-amplifying cells in the basal layer, reducing the risk of carcinogenesis. On the other hand, the protection of suprabasal cells is essential for maintenance of skin integrity.

A novel role for cornified envelope proteins in ROS protection

It has previously been shown that the stratum corneum, the outermost layer of the epidermis, also provides protection from ROS damage, for example, by production of low-molecular-weight antioxidants (Weber *et al.*, 1999). Vermeij and co-workers (2011) now provide evidence for an additional ROS defense mechanism in the stratum corneum, involving the cysteine-rich proteins of the cornified envelope (CE) (Figure 1). The investigators observed a significant quenching of singlet oxygen by CEs isolated from peeled sunburned human skin using a flash-photolysis detection method. This finding further demonstrates that the most external layers of anucleated "dead" keratinocytes provide significant protection from ROS damage by exogenous insults. Keratinocytes of the stratum corneum therefore constitute a first line of defense against ROS-induced damage from the environment.

A dual function of small proline-rich proteins: contribution to barrier function and ROS-quenching activity

Among the cornified envelope proteins, Vermeij *et al.* (2011) identified the small proline-rich protein (SPRR) family as potent antioxidants. SPRRs are 6–18 kDa in size and consist of head and tail domains rich in lysine

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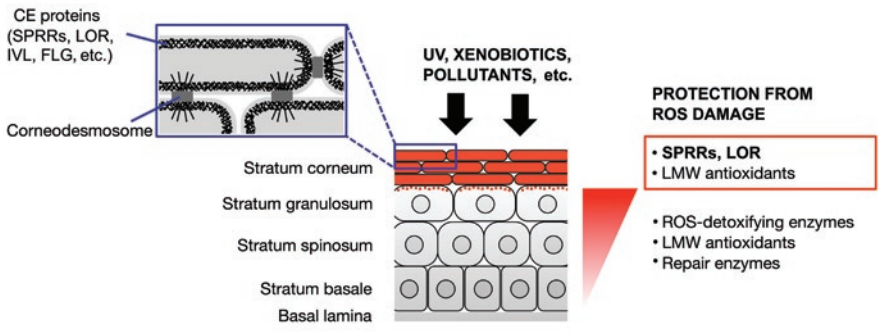

Figure 1. Reactive oxygen species (ROS) protection mechanisms of the epidermis. The epidermis is exposed to environmental insults such as UV radiation, xenobiotics, and pollutants. Multiple mechanisms protect keratinocytes from ROS damage induced by these insults. In the stratum corneum, several cornified envelope proteins, including loricrin (LOR) and small proline-rich proteins (SPRRs), function as antioxidants together with low-molecular-weight (LMW) antioxidants (indicated by red cells). In addition, a gradient of ROS-detoxifying enzymes, LMW antioxidants, and repair enzymes provides greater protection for suprabasal keratinocytes than for basal keratinocytes (indicated by the red gradient). Inset: cornified envelope proteins such as SPRRs, LOR, involucrin (IVL), and filaggrin (FLG) form a meshwork at the periphery of keratinocytes in the stratum corneum, which is cross-linked to keratin filaments. Corneodesmosomes connect the keratin filaments and the cornified envelope of stratum corneum keratinocytes. CE, cornified envelope.

and glutamine. The *SPRR* gene family comprises four subclasses (SPRR1, -2, -3, and -4), which are localized in the epidermal differentiation complex (Patel *et al.*, 2003). During late differentiation, *SPRR*s are expressed together with other genes of this complex that encode structural proteins such as loricrin, involucrin, and filaggrin. In the outermost layers of skin, these proteins are cross-linked at the cell periphery by transglutaminases; together with lipids they form the CE beneath the keratinocyte membrane (Hohl *et al.*, 1995). As an integral part of the CE, SPRRs are involved in barrier function and mechanical stability of the epidermis.

A novel function of SPRRs in ROS defense was first reported in an earlier publication from the Backendorf Laboratory (Vermeij and Backendorf, 2010). In that study, the authors showed that SPRRs are expressed by migrating keratinocytes of the epidermis in healing wounds. This was determined to be functionally important because knockdown of SPRRs inhibited keratinocyte migration *in vitro*, and this deficiency was rescued by addition of the antioxidant vitamin C (Vermeij and Backendorf, 2010). Thus, the ROS-quenching properties of SPRRs are likely to be important for normal wound repair.

In this new report, Vermeij *et al.* (2011) demonstrate that the ROS-quenching properties of SPRRs are also important in nonwounded skin, and they have extended their findings to other components of the CE. Upon overexpression of individual CE proteins in HeLa cells, SPRRs (in particular, SPRR4) were shown to be more potent antioxidants than loricrin. Their ROS-quenching activity is attributable to the high number of cysteine residues in these proteins, which directly interact with ROS and result in intermolecular disulfide bond formation. The cysteine residues in these bonds were identified in proteins of the CE by mass spectrometry after chemical modification. Surprisingly, SPRR2s but not other SPRRs were identified by this approach. Nevertheless, this analysis identified filaggrin-2 and keratinocyte proline-rich protein (KPRP) as CE proteins containing oxidation-sensitive cysteines. In addition to the cysteine content, structural differences were shown to affect the ROS-quenching potential, and an especially favorable configuration, exposing oxidation-sensitive cysteine residues, was predicted for SPRR4. Thus, SPRRs act as antioxidants in normal epidermis, contributing significantly to its antioxidant properties (Figure 1).

It has previously been reported that *SPRR4* expression is upregulated in the epidermis upon UV irradiation. This response correlated with the hyperkeratosis seen after chronic UV irradiation, and SPRR4 was specifically incorporated into fragile CEs under these conditions (Cabral *et al.*, 2001). *SPRR4* upregulation could therefore be involved in the adaptive response of the epidermis to UV radiation by maintaining CE barrier function. The novel data by Vermeij *et al.* (2011) indicate that *SPRR4* upregulation also results in increased antioxidant defense as an adaptive response to UV irradiation. The contribution of individual CE components to the ROS-quenching activity of the CE and the epidermis should be studied *in vivo*. For this purpose, knockout and transgenic mouse models of *Sprr*s (particularly *Sprr4*) should be generated in order to demonstrate the consequences of Sprr gain or loss of function on CE formation and protection from ROS damage after UV irradiation or xenobiotic treatment.

In addition, it will be important to determine the contribution of individual ROS defense mechanisms (ROS quenching by CE proteins, enzymatic ROS detoxification, and low-molecular-weight antioxidants) to the overall ROS defense in normal, wounded, UV-irradiated, and diseased skin—and to what extent these components can functionally compensate for one another. Furthermore, it would be interesting to unravel the molecular mechanisms that control individual components of the ROS defense system of the epidermis—an important prerequisite for developing novel strategies to protect stressed skin.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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